

Figure 1. Outline of protocol R.

Recovery of ds-NA takes place from the initial pellet (R-pellet), recovery of ss-NA takes place from the initial supernatant (R-sup). L₁₁, L₁₀, L₆ and L₂ are GuSCN based-buffers, SC is silica particle suspension. For details see Materials & Methods section.

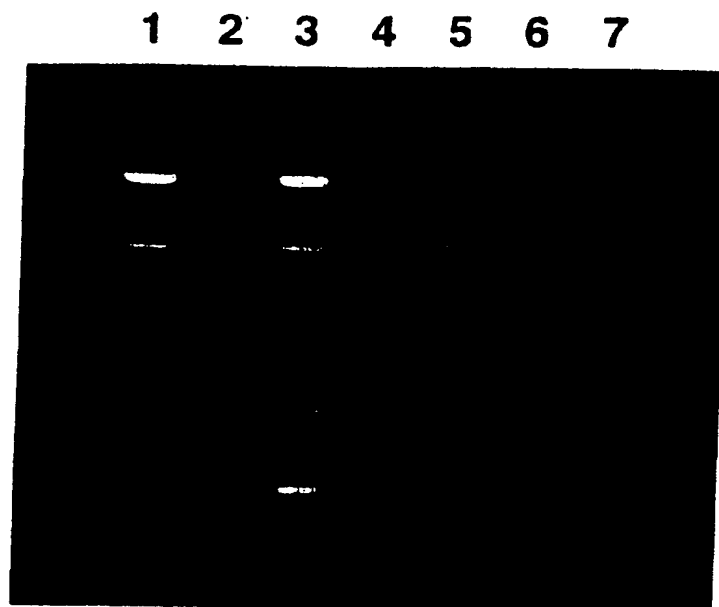


Figure 2. Separation of ds-DNA and ss-DNA.

NA was purified (in duplicate) by protocol R from a mixture of ds-DNA (phage lambda, HindIII digest, 1 μ g) and ss-DNA (phage M13 DNA, 500ng). Final elutions were in 50 μ l TE and 25 μ l were electrophoresed through a 1% agarose gel (containing ethidiumbromide) which was subsequently photographed under UV-illumination. Lane 1, 100% recovery marker for ds-DNA fragments; lane 2, 100% recovery marker ss-DNA; lane 3, 100% recovery marker mixture ds-DNA/ ss-DNA. Lanes 4 and 5, output protocol R-pellet; lanes 6 and 7, output protocol R-sup.

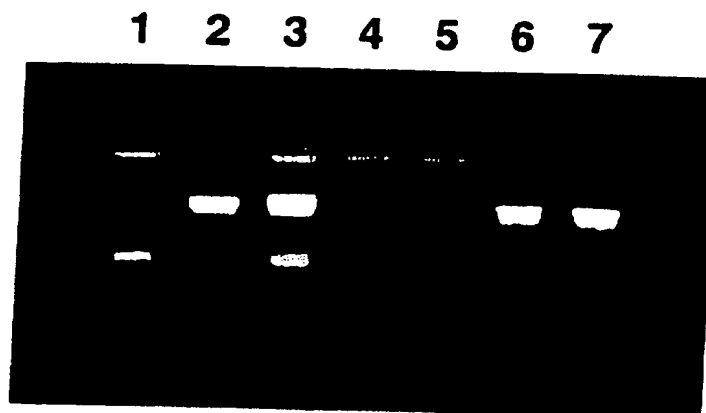


Figure 3. Separation of ds-RNA and ss-RNA.

NA was purified (in duplicate) by protocol R from a mixture of ds-RNA (Rotavirus ds-RNA) and ss-RNA (phage MS2 RNA, 800ng). Final elutions were in 50 μ l TE and 25 μ l were electrophoresed through a 1% agarose gel (containing ethidiumbromide) which was subsequently photographed under UV-illumination. Lane 1, 100% recovery marker for ds-RNA fragments; lane 2, 100% recovery marker ss-RNA; lane 3, 100% recovery marker ds-RNA/ss-RNA mixture. Lanes 4 and 5, output protocol R-pellet; lanes 6 and 7, output protocol R-sup.

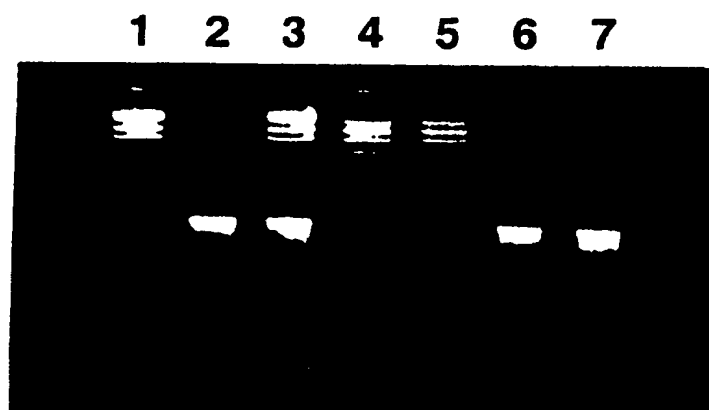


Figure 4. Separation of ds-DNA and ss-RNA.

NA was purified (in duplicate) by protocol R from a mixture of ds-DNA (750 ng phage lambda digested with hindIII) and ss-RNA (phage MS2 RNA, 800ng). Final elutions were in 50 μ l TE and 25 μ l were electrophoresed through a 1% agarose gel (containing ethidiumbromide) which was subsequently photographed under UV-illumination. Lane 1, 100% recovery marker for ds-DNA; lane 2, 100% recovery marker for ss-RNA; lane 3, 100% recovery marker for ds-DNA/ss-RNA mixture. Lanes 4 and 5, output protocol R-pellet; lanes 6 and 7, output protocol R-sup.

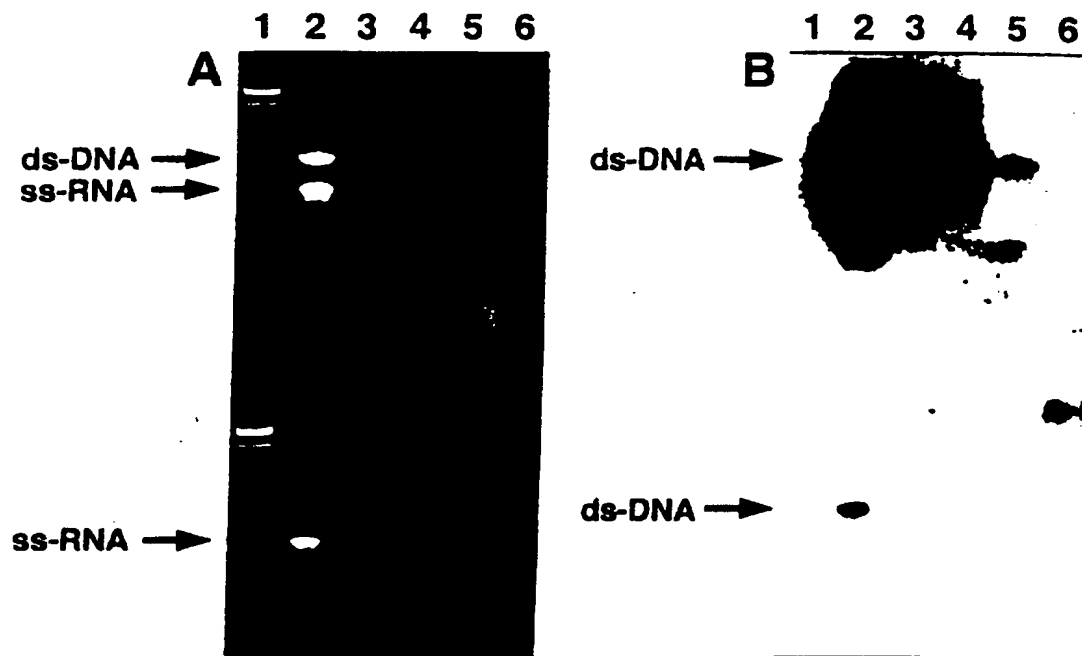


Figure 5. Separation of ds-DNA and ssRNA.

NA was purified by protocol R-sup from a mixture of ds-DNA (1000 ng linearized pHC624, 2kb) and ss-RNA (phage MS2 RNA, 800ng). Final elution was in 50 μ l TE, and 25 μ l or tenfold serial dilutions of the ss-NA fraction were electrophoresed through a 1% agarose gel (containing ethidiumbromide) which was subsequently photographed under UV-illumination.

Panel A: Upper row: lane 1, HindIII digested phage lambda DNA; lane 2, 100% recovery marker for ds-DNA and ss-RNA and serial tenfold dilutions thereof (lanes 3-6). Bottom row, output of protocol R-sup (lane 2) and tenfold serial dilutions (lanes 3-6).

Panel B: Ds-DNA was subsequently transferred to a nitrocellulose filter and hybridized with a 32 P-labelled probe homologous to input ds-DNA.

ds-DNA and ss-RNA are indicated.

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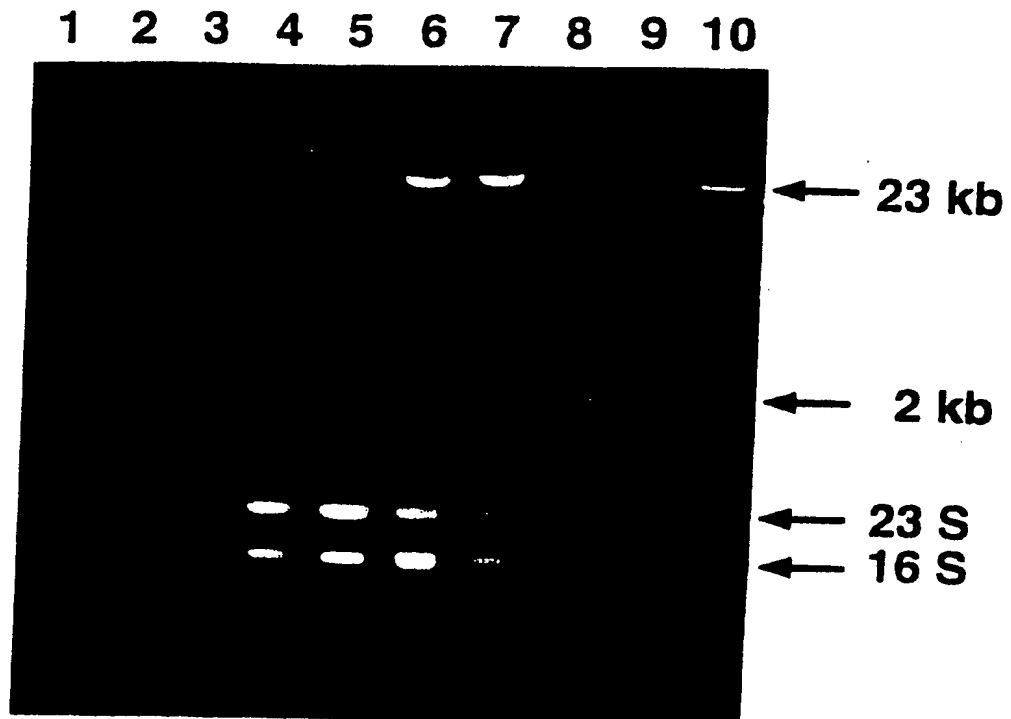


Figure 6. Separation of genomic DNA from ss-RNA.

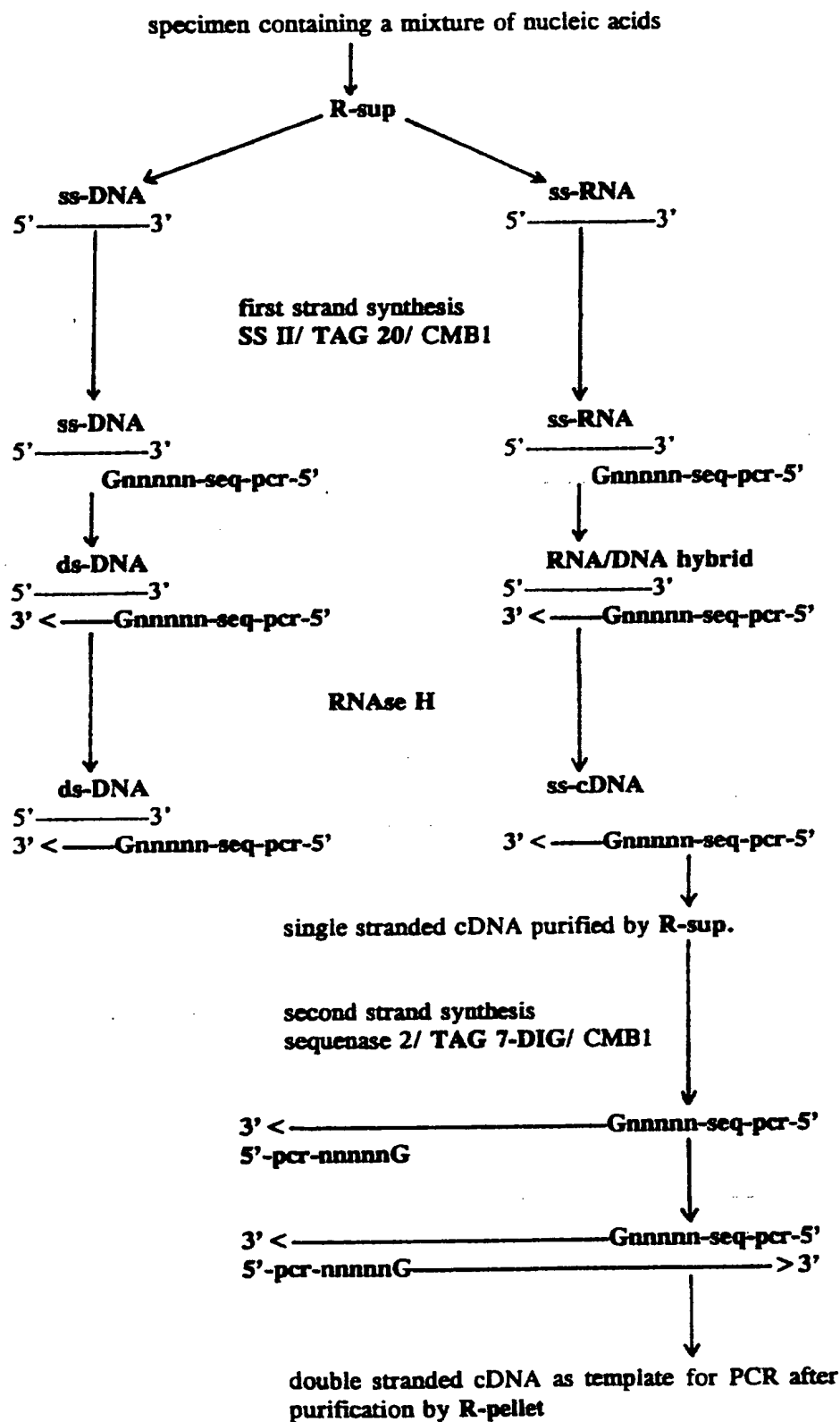
How to deal with trapping of ss-RNA. *E. coli* bacteria were directly used as input material for duplicate extractions by protocol R (lanes 6 and 7, R-pellet; lanes 8 and 9, R-sup). Alternatively, total NA was first purified by protocol Y using diatoms as NA-carrier (which causes shearing of genomic DNA). The purified nucleic acids were subsequently used as input for protocol R (lanes 2 and 3, R-pellet; lanes 4 and 5, R-sup). Final elutions were in 50 μ l TE and 25 μ l were electrophoresed through a 1% agarose gel (containing ethidiumbromide) which was subsequently photographed under UV-illumination.

Marker lanes 1 and 10 500 ng phage lambda DNA, HindIII digested).

23S and 16S rRNA, and ds-DNA molecular weight markers (23kb and 2.0 kb) are indicated.

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Figure 7 Outline of the procedure



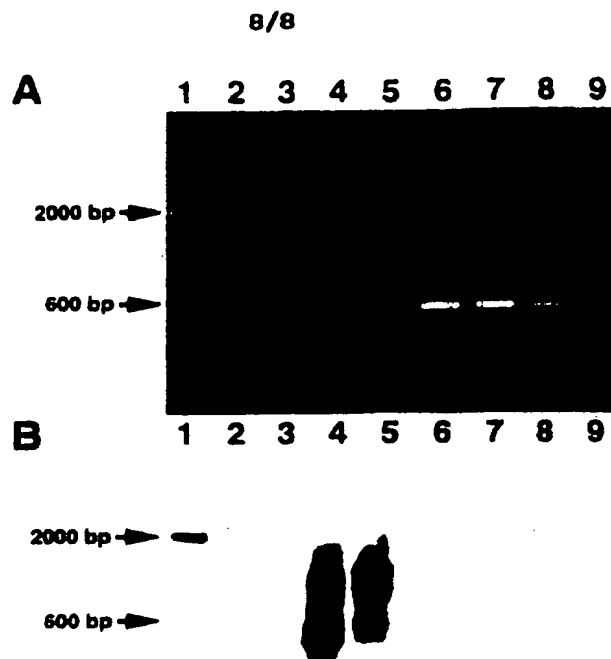


Figure 8

Single-stranded nucleic acid was purified from samples containing HIV-1 RNA and TE (negative control) by protocol R-sup. and subsequently amplified with the non-selective RT-PCR.

Panel A: lane 1, 100 bp DNA ladder; lanes 2 and 3 negative extraction controls; lanes 4 and 5 non-selectively amplified HIV-1 RNA; lanes 6, 7, 8 and 9 600, 60, 6 and 0 molecules resp. of pHCrec (positive PCR control).

Panel B: Southern blot hybridization with ^{32}P -labelled HIV-1 probes (containing the GAG, POL and ENV genes of HIV-1) of the samples shown in panel A. After overnight hybridization at 65 °C in 6 x SSC, 0.1 % SDS, 10 % Dextran Sulphate and 50 $\mu\text{g/ml}$ salmon sperm DNA, the filter was subsequently washed under high stringency conditions with 0.1 SSC/0.1% SDS at 65°C, and autoradiographed on X-ray film for two hrs. at -70°C. This experiment showed that most of the bands visible on the ethidiumbromide stained agarose gel originated from the HIV-1 genome.